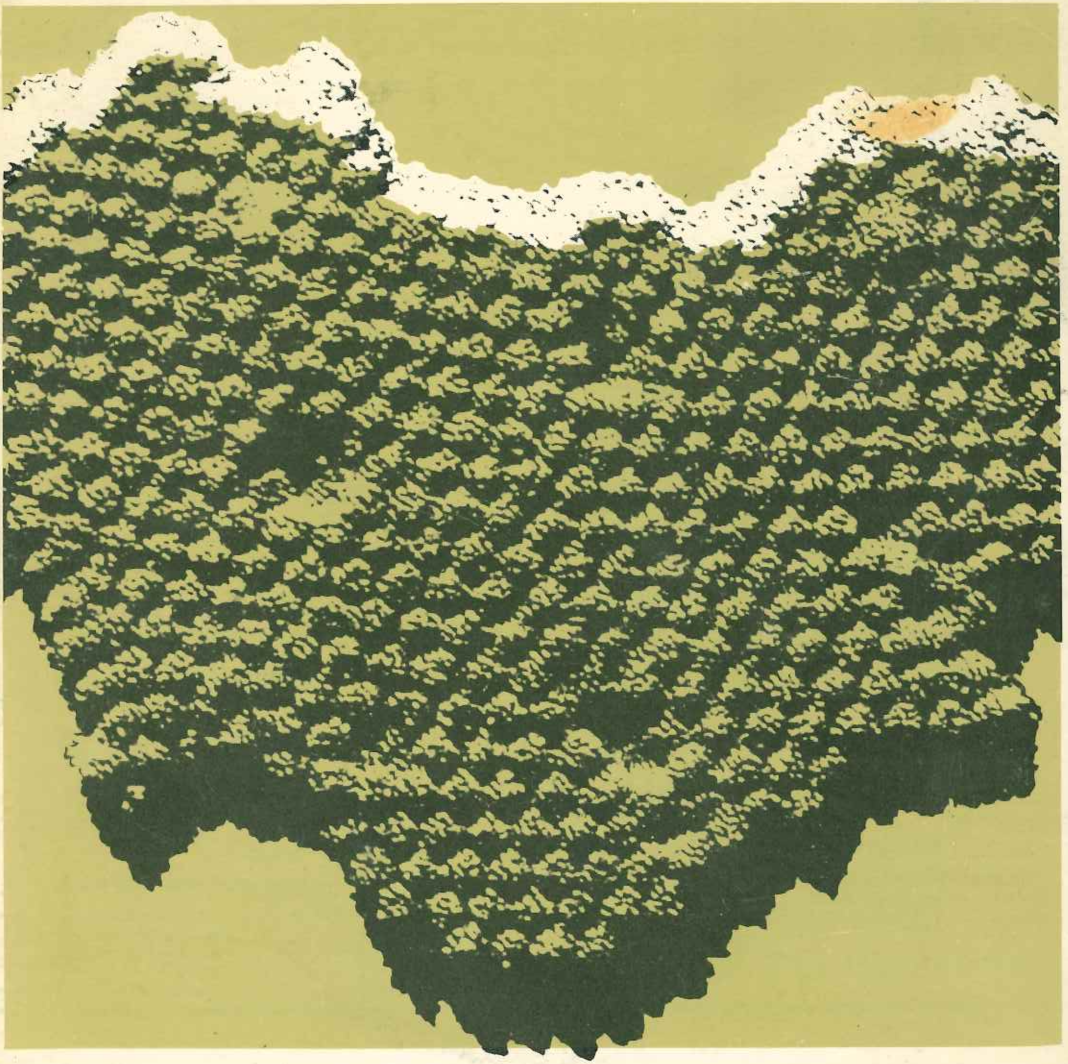
# Beaded Sepharose®
# 2B - 4B - 6B

## covering the whole field of agarose gel filtration



## Cover

Electron photomicrograph of crystallized satellite tobacco necrosis virus.
Linear magnification 360,000 ×.
Photo by Dr. S. Höglund, Biochemical Institute, Uppsala University.

Sepharose® is the registered trade mark of spherical agarose gel beads manufactured by Pharmacia Fine Chemicals AB.

Gel filtration, the chromatographic technique which is also often called gel chromatography or molecular (particle) sieve chromatography, has been comprehensively reviewed (1, 2) and is also described in detail in the Pharmacia Fine Chemicals' booklet: "Sephadex — gel filtration in theory and practice".

The three Sepharose types, 2B, 4B and 6B cover the whole range of gel filtration of high molecular weight proteins and polysaccharides, nucleic acids and viruses. All three gels fractionate in a range above Sephadex® G-200 and therefore form a valuable complement to the series of Sephadex types.

Sepharose is supplied as a dense, diethyl pyrocarbonate-sterilized, suspension of swollen beads in distilled water containing 0.02 % sodium azide as a bacteriostatic agent.

| Type | Approx. agarose conc. % | Particle size in wet state | Approx. exclusion limits, MW | |
|---|---|---|---|---|
| | | | polysaccharides | proteins |
| Sepharose 2B | 2 | 60—250 μ | $20 \times 10^6$ | $40 \times 10^6$ |
| Sepharose 4B | 4 | 40—190 μ | $5 \times 10^6$ | $20 \times 10^6$ |
| Sepharose 6B | 6 | 40—210 μ | $1 \times 10^6$ | $4 \times 10^6$ |

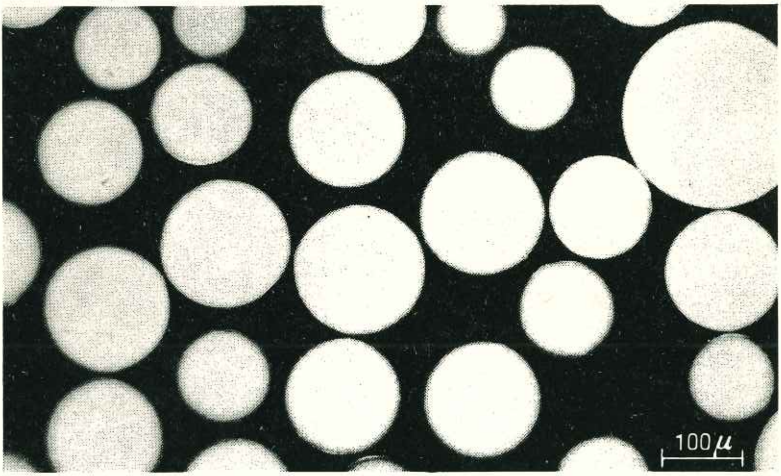


**Fig. 1** Photomicrograph of Sepharose 2B.

# Preparation of Sepharose

The agarose used to manufacture Sepharose is produced from selected agar by the method of *Hjertén* (3, 4).

According to *Araki* (5) agar is a complex of agarose and agaropectin. Agaropectin, a polysaccharide which contains sulphate and carboxyl groups, imparts ion exchange properties to the agar. These properties cause undesirable side-effects in gel filtration.

Agarose is a linear polysaccharide which consists of alternating residues of D-galactose and 3,6 anhydro-L-galactose units. It has at least the same good gelling properties as agar. The gelling is attributed to hydrogen bonding. Because of their hydrophilic nature and the nearly complete absence of charged groups agarose gels, like dextran gels, cause very little denaturation and adsorption of sensitive biochemical substances.

Sepharose is prepared from the agarose according to a modification of the method of *Hjertén* (6). The microscopic appearance of Sepharose is shown in fig. 1.

The quality of Pharmacia Fine Chemicals' agarose preparations is strictly controlled. The absence of agaropectin is routinely established by stringent analytical procedures.

The absence of cytotoxic substances, an important consideration when chromatographing viruses, cell particles etc., has been established.

# Gel filtration on Sepharose

The Sepharose types are, by virtue of their greater porosity, an important complement to the series of Sephadex types. Whereas Sephadex allows excellent fractionation of macromolecules, such as globular proteins, of dimensions corresponding to molecular weights up to 600,000, Sepharose may be used to separate molecules and particles up to molecular (particle) weights of several millions.

As early as 1961 *Polson* (7) introduced granulated agar gels for gel filtration in the high molecular weight range. The practical difficulties associated with the granulated form and the presence of charged agaropectin prevented widespread use of this bed material. The introduction of agarose gels by *Hjertén* (8) was a considerable improvement, but the real break-through did not occur until 1964 when *Hjertén* (6) and *Bengtsson* and *Philipson* (9) succeeded in preparing these gels in bead form. *Joustra* (10) has shown that beaded agarose gels have a greater separating power than granular gels of comparable percentage agarose. Thus beaded agarose gels have not only superior flow properties but also higher resolving power.

The flow characteristics of Sepharose are illustrated in fig. 2. The fractionation ranges of Sepharose are presented in the form of selectivity curves for both dextrans and proteins (see fig. 3 and fig. 4). The slope of the selectivity curve, $K_{av}$ versus log M, is a measure of the separating power: the steeper the curve, the greater the differences in $K_{av}$ values between substances to be separated.

By extrapolation of the selectivity curves the exclusion limits may be estimated at $1 \times 10^6$, $5 \times 10^6$ and $20 \times 10^6$ for Sepharose 6B, 4B and 2B, respectively, for polysaccharides. The exclusion limits for proteins can be obtained in a similar way; $4 \times 10^6$ for Sepharose 6B, $20 \times 10^6$ for 4B and $40 \times 10^6$ for 2B.

From figs. 3 and 4 it can be seen that the selectivity curves for Sepharose 6B and 4B and Sephadex G-200 overlap to some extent. The selectivity curve for Sephadex G-200 is steeper than those for Sepharose 4B and 6B and Sephadex G-200 is, therefore, the gel of choice for fractionations up to about 600,000 in molecular weight. Sepharose 6B, however, is a much more rigid gel and can be used in the upper fractionation range of Sephadex G-200 when high flow rate is more important than resolution.

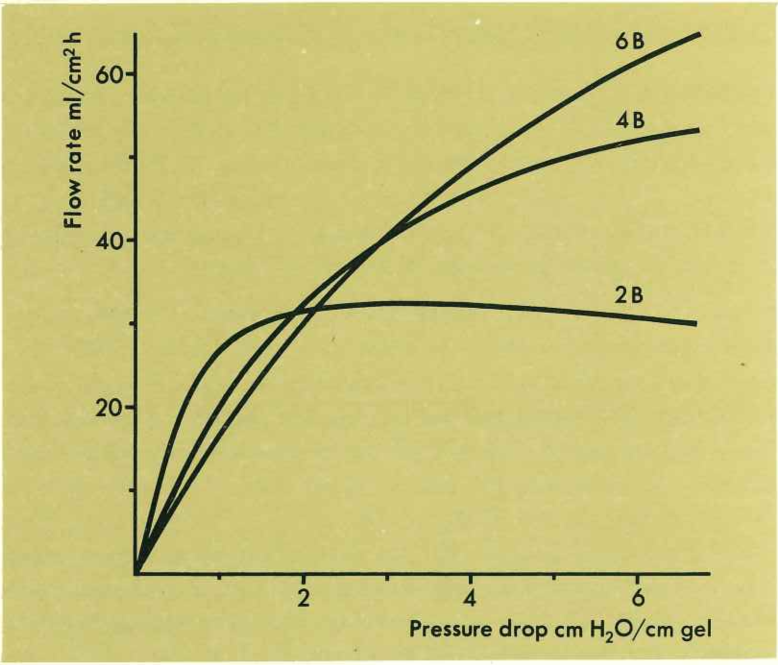


**Fig. 2** Relationship between pressure drop and flow rate. The data were obtained from experiments with columns of 5.0 cm diameter. Fixed bed heights of 10 cm were used. The pressure was increased at intervals of one hour.

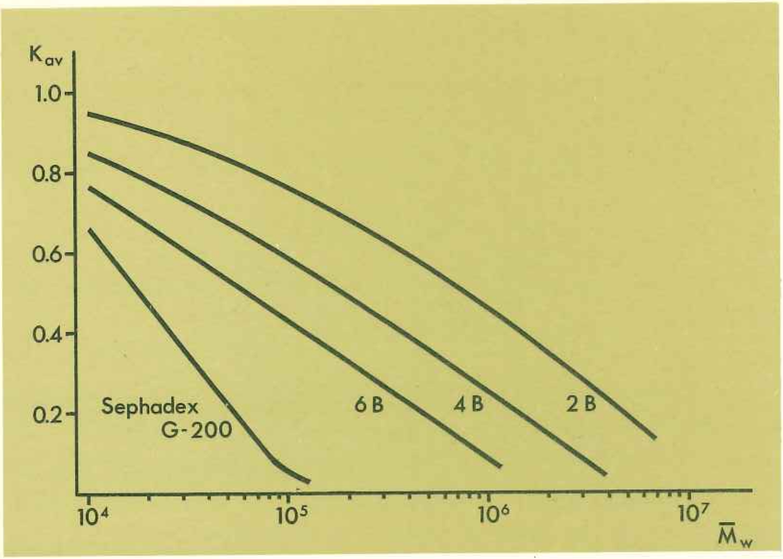


**Fig. 3** The dextran molecular weight selectivity curves of Sephadex G-200, Sepharose 6 B, Sepharose 4 B and Sepharose 2 B. The dextran preparations used to obtain the plotted data were specially prepared fractions of very narrow molecular weight distribution.

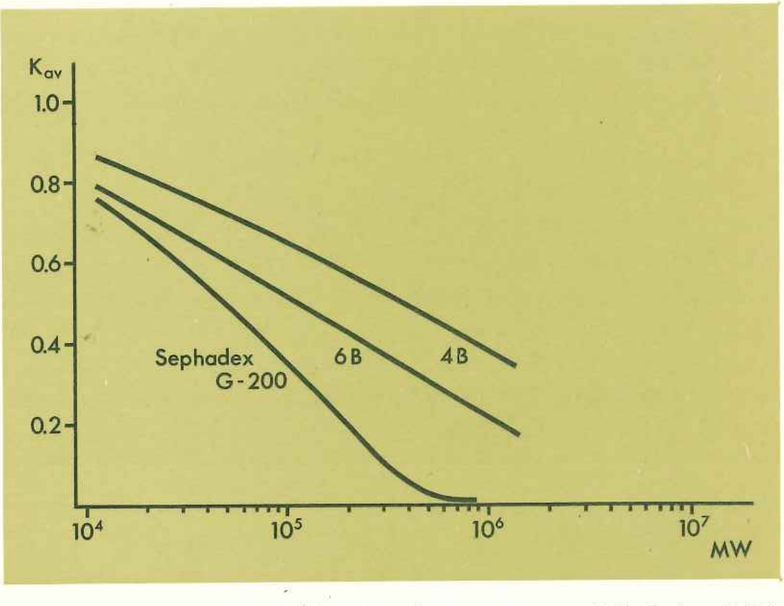


**Fig. 4** The protein molecular weight selectivity curves of Sephadex G-200, Sepharose 6 B, Sepharose 4 B determined with ribonuclease, ovalbumin, transferrin, glucose oxidase, thyroglobulin and $\alpha$-crystallin.

# Applications

The applications of Sepharose 2B and 4B have been concentrated in the study of viruses, bacteriophages and nucleic acids, especially those of viral origin. Sepharose 6B, however, is more suitable for the chromatography of high molecular weight proteins. The examples given below illustrate the fields of application of the three Sepharose types.

*Öberg* and *Philipson* (11) have used Sepharose 2B to separate KB-cell nucleic acids, from each other and from viral RNA (Fig. 5). *Erikson* and *Gordon* (12) have reported the application of Sepharose 4B in separating the so-called replicative intermediate form of phage-RNA from *E. coli* r-RNA. *Höglund* (13) used Sepharose 4B to demonstrate the homogeneity of tail fibers from T2 phages during studies on the interaction of the T2 phage and its IgG and IgM antibodies.

*Bengtsson* and *Philipson* (14) have demonstrated the value of Sepharose 2B for gel filtration of viruses (Fig. 6). *Kingsbury* (15) used Sepharose 2B for the purification of viral RNA during studies on Newcastle disease virus. *Russell* and *Osborn* have used Sepharose 2B in the study of *Tubifex* haemoglobin (haemoglobin oligomers) (16). *Malchow et al.* (17) have used Sepharose 4B for the study of bacterial lipopolysaccharides. *Loeb* (18) has presented gel filtration patterns of deoxyribonucleoprotein from calf thymus and rat liver nuclei on Sepharose 4B. *Pettersson et al.* (19) used gel filtration on Sepharose 6B as the final purification step in the preparation of adenovirus type 2 fibre antigen. *Joustra* (10) has used Sepharose 6B in the purification of thyroglobulin (Fig. 7).

*Killander et al.* (20) have described the separation of plasma macroglobulins on columns of beaded 3.5 % agar gel. This type of separation can also be accomplished on columns of Sepharose 4B.

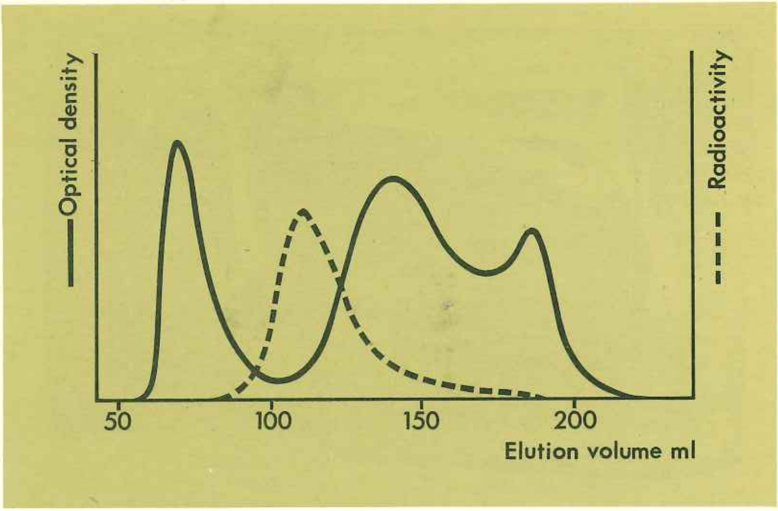


**Fig. 5** Separation of a mixture of KB-cell nucleic acids and $^{32}P$-labelled poliovirus RNA on Sepharose 2B. The first peak contains KB-cell DNA, followed by poliovirus RNA, KB-cell r-RNA and KB-cell s-RNA.
Bed dimensions: 2.1 × 60 cm. Eluant: 0.002 M sodium phosphate buffer, pH 6.0 and 0.001 M magnesium chloride. Flow rate: 2 ml/cm² h. Sample volume: 1 ml.

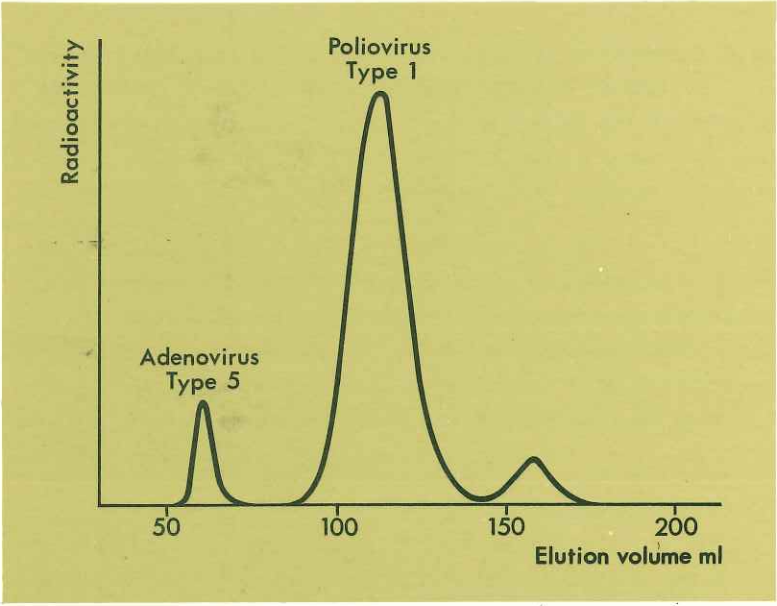


**Fig. 6** Separation of $^{32}P$-labelled adenovirus and poliovirus on Sepharose 2B. Bed dimensions: 2.1 × 56 cm. Eluant: 0.002 M sodium phosphate buffer, pH 7.2 and 0.15 M sodium chloride. Flow rate: 2 ml/cm² h. Sample volume: 1 ml.

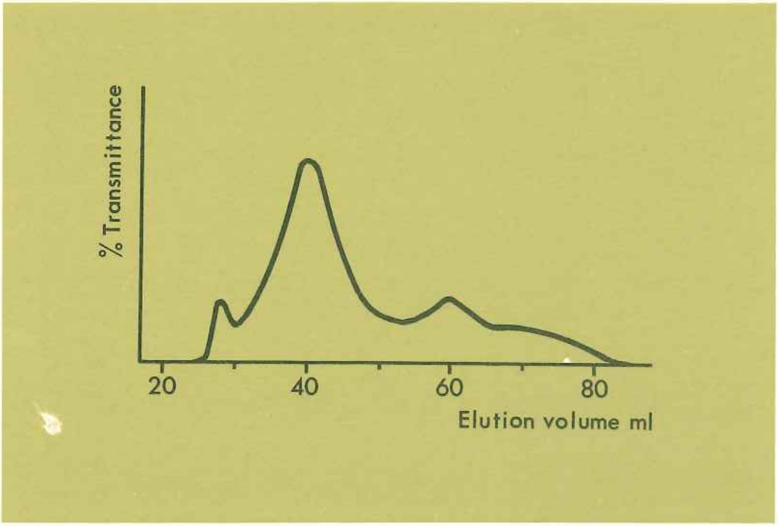


**Fig. 7** Purification of commercially available thyroglobulin by gel filtration on Sepharose 6B.
Bed dimensions: 1.5 × 50 cm. Eluant: 0.1 M Tris-HCl buffer, pH 8.0 and 0.5 M NaCl. Flow rate: 8 ml/cm$^2$ h. Sample volume: 1 ml.

# Preparation of Sepharose beds

Sepharose beds may be packed in the same way as Sephadex as outlined in the Pharmacia Fine Chemicals' booklet: "Sephadex — gel filtration in theory and practice". For Sepharose gel filtration the Laboratory Columns and the Flow Adaptors are recommended. These are described in the Pharmacia Fine Chemicals' booklet: "Sephadex laboratory columns".

The suspension of Sepharose, as provided, is too dense to be poured into a column. It should first be diluted to a consistency which allows easy escape of air bubbles. The suspension contains sodium azide (0.02 %) to suppress microbial growth. This bacteriostatic agent should be washed out before substances sensitive to it are exposed to the gel.

The void volume of a Sepharose bed can be measured by running a sample of DNA or Blue Dextran 2000 through the column. The UV-elution profiles of this coloured dextran are depicted in fig. 8. Blue Dextran 2000 contains some material of very high molecular weight which is excluded from Sepharose. The leading peaks which contain these very large molecules indicate the void volume.

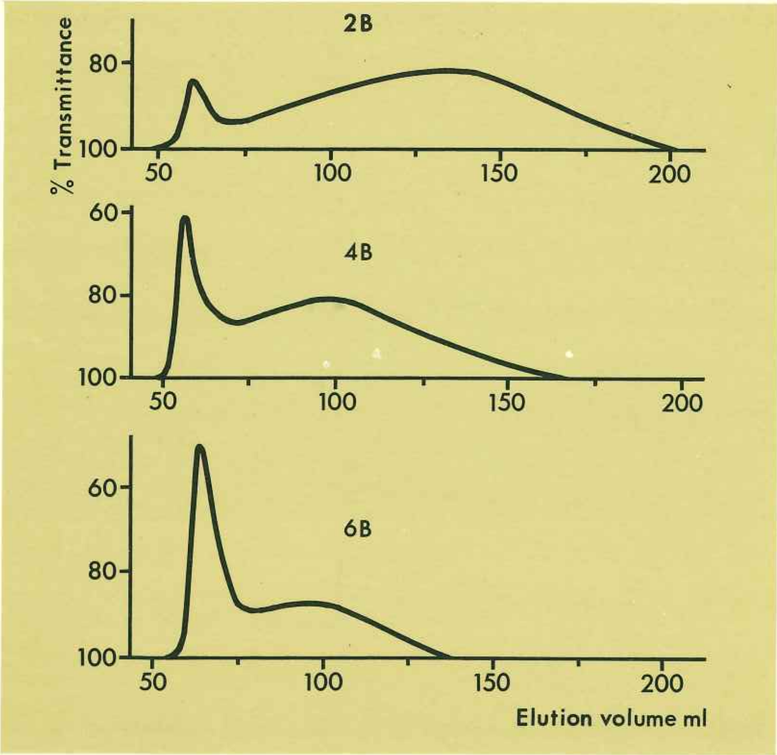


**Fig. 8** The elution profiles of Blue Dextran 2000 on columns of Sepharose 6B, 4B and 2B. In each case the leading peak indicates the void volume.

# Chemical stability

Since there are no cross-linkages of a covalent nature in Sepharose, stability of the gel beads can only be expected within certain limits. The pH of the eluant should not be above 9 or below 4, and temperatures below 0° C and above 40° C should be avoided. However, Sepharose does not appear to be affected by rather high concentrations of salt and urea. Exposure to 1 M sodium chloride and 2 M urea have been shown not to change the properties of Sepharose noticeably.

# Storage

When Sepharose is not in use it should be stored in the swollen state and protected from microbial contamination by a suitable disinfectant. At the laboratories of Pharmacia Fine Chemicals 0.02 % sodium azide is used for this purpose. From other laboratories the use of butanol (0.5 %) and chloroform (saturated solution) has been reported. Other antimicrobial agents should only be used if they are known not to affect agarose gels.

# Availability and orders

Sepharose is available in 1 litre packs only. It can be ordered through all Pharmacia subsidiaries and representatives. If there is none in your country, please write to Pharmacia Fine Chemicals, Uppsala, Sweden.

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# APPENDIX TO SEPHAROSE BOOKLET

## Flow rate through Sepharose® beds

Measurements of the flow rates through beds of Sepharose have been made in our laboratories. Each of the three types of Sepharose, 2B, 4B and 6B has been tested in columns of our laboratory series at different operating pressures (fig. 1 - 6). These data complete the data presented in fig. 2 of the Sepharose booklet.

Sepharose was packed into the columns and equilibrated overnight at the lowest operating pressure tested. The eluant was 0.9% NaCl solution containing 0.2% $NaN_3$ as a preservative. The flow rate was measured at several operating pressure allowing 2 hr for stabilization between each pressure increase. Note that the pressure was measured across the gel bed and that the pressure drop across the capillary tubing connected to the column was corrected for.

Tests of the long-term stability of the flow rate through Sepharose 2B (K 15/90 column, bed height 84 cm) showed that the flow rate remains constant to within 10% over at least six weeks. It should be noted that, if Sepharose beds are allowed to stand without eluant flowing through them, the flow rate subsequently obtained may be reduced by 10%.

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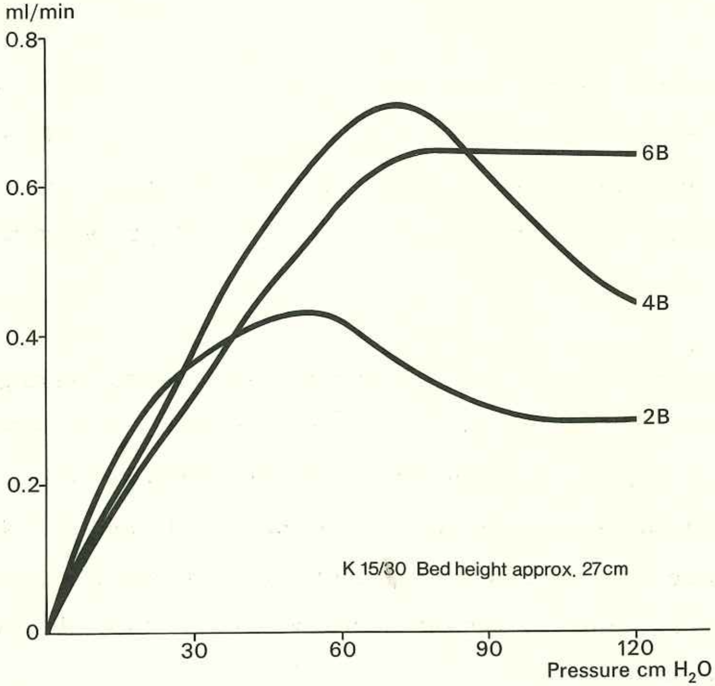
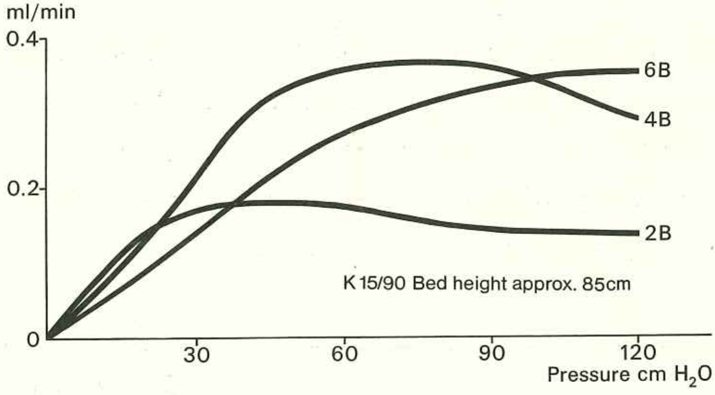

ml/min
0.8
0.6
0.4
0.2
0
30
60
90
120
Pressure cm H₂O
6B
4B
2B
K 15/30 Bed height approx. 27cm


ml/min
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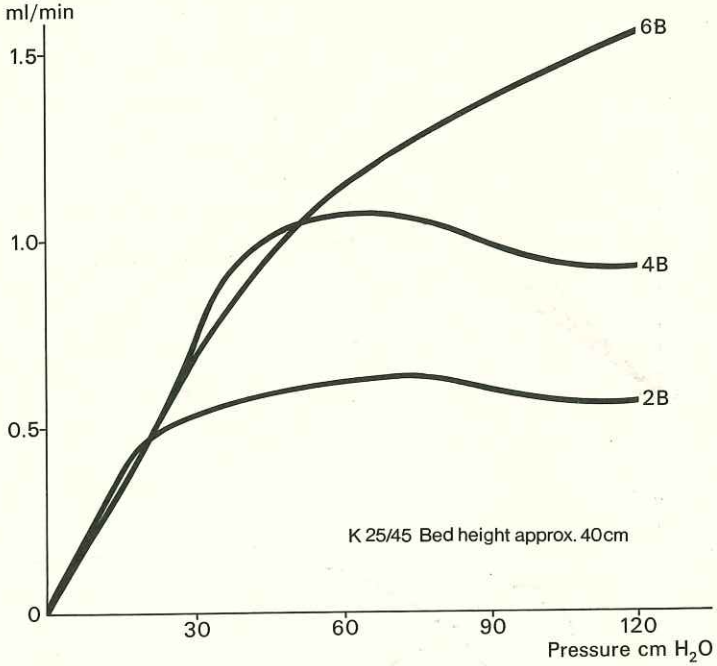
0.2
0
30
60
90
120
Pressure cm H₂O
6B
4B
2B
K 15/90 Bed height approx. 85cm


ml/min
1.5
1.0
0.5
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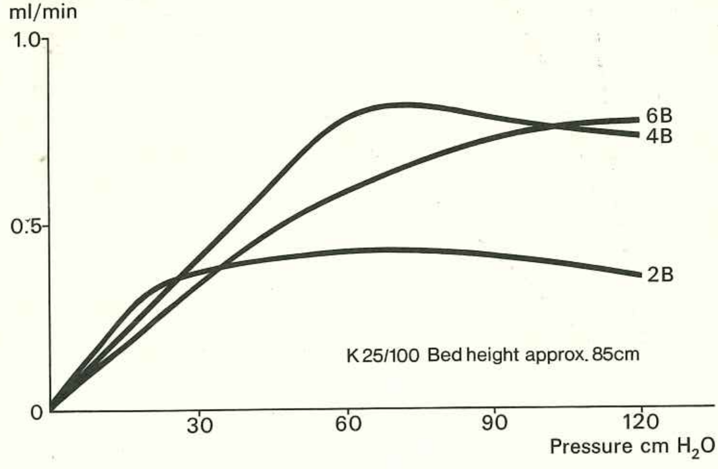
30
60
90
120
Pressure cm $H_2O$
6B
4B
2B
K 25/45 Bed height approx. 40cm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Pressure cm $H_2O$
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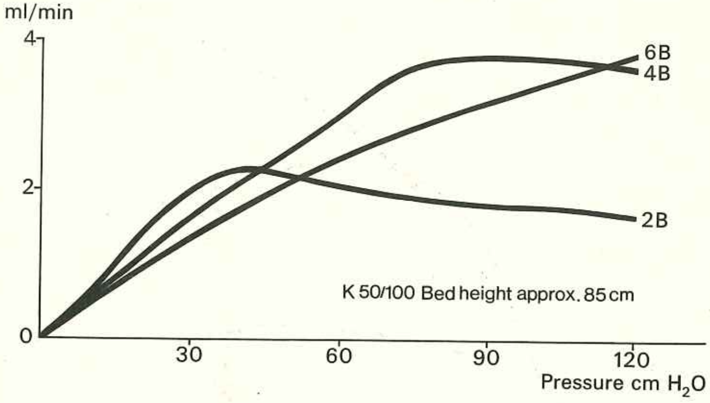
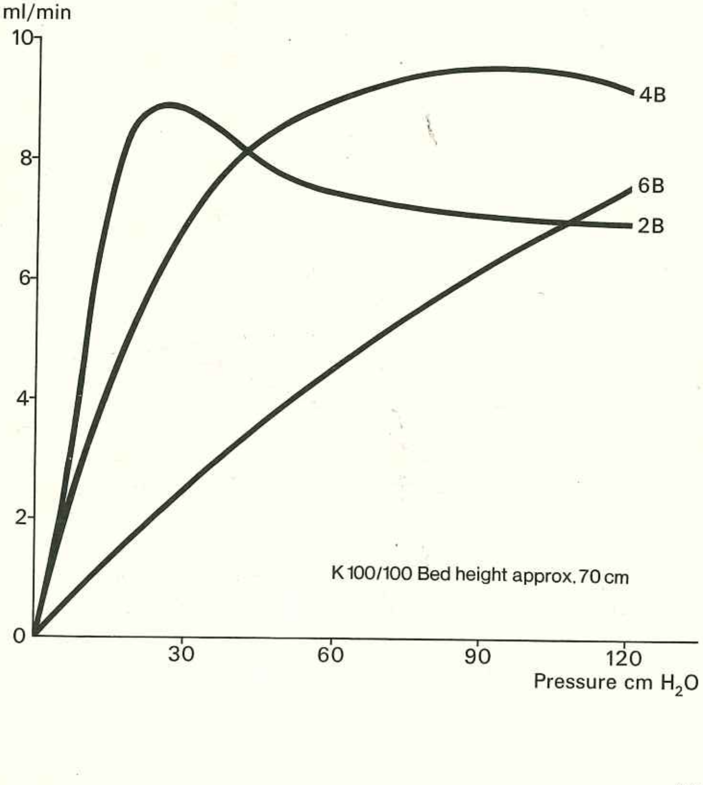
K 25/100 Bed height approx. 85cm





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